

Caulobacter Electroporation Transformation:

Materials Needed:

Electrocompetent Cells	Glass beads
PYE Plates with Chloramphenicol (2 ug/mL)	Pipets
PYE Media	Electroporation Cuvettes
	Kimwipes
	Inoculation Loop

Protocol (estimated time 2-3 days):

1. Thaw your electrocompetent cells and plasmid on ice. Additionally, chill two electroporation cuvettes on ice.
 - a. Cuvettes are placed on ice to prevent cell shock when transferred to a new environment
2. After about 20 minutes of chilling the electroporation cuvettes, pipet in 1 μ L of plasmid DNA (50-100 μ g) and 50 μ L of cells.
 - a. The cells are either allowed to rest with the DNA for 15 - 30 minutes or are taken directly electroporated.
 - i. Some protocols recommend resting with DNA, whereas others recommend electroporating right away.
3. Wipe down the sides of the electroporation cuvette with a kimwipe and place it into the electroporation machine.
 - a. Wiping down the outside prevents sparks from forming/damaging the machine.
4. Electrocute the cells 1800 kV, 200ohms, 25 μ F with a time constant less than 4ms.
 - a. No arcing should occur.
5. Gently remove the electroporation cuvette from the machine and pipet in 1000 μ L PYE media. Pipet the cuvette contents into eppendorf tube
6. Incubate tube, shaking, for 2 hours at 30°C
7. Repeat steps 4 through 6 with a no plasmid control
8. Spin down contents of eppendorf tube at 8000 rpm for 2 minutes
 - a. Remove 900 μ L of supernatant
 - b. Resuspend the cell pellet in the remaining supernatant
9. Plate the cell suspension on a PYE plates and incubate for 2-3 days at 30°C
10. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up all the TSB and cells
 - a. If there is growth on the negative controls of electroporated cells without plasmid on the antibiotic plates, then the transformation was unsuccessful. This may either be due to contamination or a poor concentration of antibiotic.

References

1. Williams, P., Ketley, J., & Salmond, G. (Eds.). (1998). *Bacterial Pathogenesis*. London, UK: Academic Press.